

Research Article

Can Planktonic Organisms be Useful for Determining the Drowning Depth in **Fatal Diving Accidents?**

Ölümcül Dalış Kazalarında Boğulma Derinliğinin Belirlenmesinde Planktonik Organizmalar Yardımcı Olabilir mi?

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Abstract: Objective: Although the triggering factors are various, most of the fatal diving accidents result in drowning. If drowning depth is known, it will contribute the clarification of underlying reasons of the accidents. In this experimental study, it has been investigated whether planktonic organisms can be used to determine the drowning depth in diving accidents. Materials and Methods: Planktonic organisms in water samples taken from the surface and 30 meters depth of Marmara Sea were investigated. In addition, rats in one of the two groups of 8 Wistar Albino species were drowned in the water sample obtained from surface and rats in the second group were drowned in the water sample obtained from 30 meters depth at a pressure of 4 ATA in a hyperbaric chamber and planktonic organism was investigated in the lungs of drowned rats. Results: In the autopsies planktonic organism was not found in the lungs of rats. It was observed that the lungs of the rats that drowned at 4 ATA were more bulging and bleeding. The absence of a planktonic organism in the lungs was thought to be due to the fact that the rats aspirated a very small amount of water and the plankton density in the samples was low. Conclusion: The determination of seasonal and daily migration of planktonic organisms to particular depths at dive sites can help to determine the depth of drowning in fatal diving accidents. However, in this experimental study, no planktonic organism was detected in the lungs of drowned rats.

Keywords: Diurnal Migration, Diving Accidents, Drowning, Planktonic Organisms.

Öz: Amaç: Tetikleyici neden farklı olsa da ölümcül dalış kazalarının büyük bir bölümü boğulma ile sonuçlanmaktadır. Boğulmanın gerçekleştiği derinliği bilmek, kaza nedenlerinin aydınlatılmasına önemli katkıda bulunabilir. Bu deneysel çalışmada, ölümcül dalıs kazalarında, boğulma derinliğinin tespitinde planktonik organizmaların kullanılıp kullanılamayacağının tespiti amaçlanmıştır. Gereç ve Yöntem: Bunun için Marmara Denizi'nde yüzeyden ve 30 metre derinlikten alınan su örneklerindeki planktonik organizmalar incelenmiştir. Ayrıca Wistar Albino türü 8 bireyden oluşan iki gruptan ilkinde bulunan sıçanların yüzeyden alınan su örneği içinde ve diğer grupta bulunan sıçanların ise 30 metre derinlikten alınmış su örneği içinde, bir basınç odasında 4 ATA basınç altında boğulmaları sağlanmış ve boğulan sıçanların akciğerlerinde planktonik organizmalar araştırılmıştır. Bulgular: Sıçanların boğulma sonrası yapılan otopsilerde akciğerlerinde planktonik organizmaya rastlanmamış, 4 ATA basınç altında boğulan gruptaki sıçanların akciğerlerinin daha şişkin ve kanamalı olduğu gözlemlenmiştir. Akciğerlerde planktonik organizmaya rastlanmaması, sıçanların çok az miktarda sıvı aspire etmesinden ve örnekteki plankton yoğunluğunun düşüklüğünden kaynaklanmış olabileceği düşünülmüştür. Sonuç: Dalış bölgelerindeki planktonik organizmaların, belirli derinliklere yaptıkları mevsimsel ve günlük göçlerinin belirlenmesi, ölümle sonuçlanan dalış kazalarında boğulma derinliğinin tespitine yardımcı olabilir. Ancak bu deneysel çalışmada boğulan sıçanların akciğerlerinde planktonik organizma tespit edilememistir.

Anahtar kelimeler: Diurnal Göç, Dalış Kazası, Boğulma, Planktonik Organizmalar.

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Conflict of Interest

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Ethical Declaration

Bu makale "Farklı Derinliklerde Boğulan Sıçanların Akciğerlerde Tespit Edilen Planktonik Organizmalar Yardımıyla Boğulma Derinliği Tespit edilebilir mi?" isimli uzmanlık tezinin yeniden düzenlenmesi ile oluşturulmuştur.

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1. Introduction

The first dives date back as far as 5000 years from today, although it is not known for certain when humankind first dived into the water holding its breath. It is known that the first diving trials were conducted for the purpose of extracting high commercial value goods such as pearls, sponges and coral from shallow waters. The Greek historian Herodotus mentions that Xerxes, King of Persia, commissioned a father-daughter diver named Scyllias and Cyana to find a sunken treasure in the fifth century BC (1). Today, it is possible to dive for military or professional purposes up to 300-400 meters with suitable gas mixtures, methods and equipment (2).

According to World Health Organization data, 9% of all injured accidents worldwide are caused by drowning, among the top ten causes of death in the 1-24 age range, and 372,000 people drown each year (3). Because diving activity takes place in a different environment than physiological conditions, events resulting in injury or death occur, although the causes leading to the event are a wide variety, in most of the accidents the ultimate cause of death is stated to be drowning (4). In a study conducted in our country, drowning was reported as the most common cause of death in fatal diving accidents (5).

Drowning begins with involuntary breathing following a period of breatholding while in the water. Following this, when the fluid reaches the oropharynx or larynx, laryngospasm develops reflexively. However, if the person wants to breathe, either only a small amount of fluid is aspirated into the lungs due to laryngospasm, or there is no fluid passage to the lungs at al. As a result of the respiratory failure, while the oxygen levels in the alveoli and blood drop and hypoxemia develop, carbon dioxide levels rise and hypercarbia and acidosis develop. Although there is a significant increase in respiratory movements over time, ventilation cannot occur due to obstruction at larynx level and being in water environment. Due to the falling oxygen level in the blood, the oxygen going to the larynx muscles is also reduced and energy production in the muscles cannot take place. Therefore, laryngospasm is eliminated and some fluid begins to enter the person's lung after a while (6).

In a fatal diving accident, having an idea of the depth at which the drowning took place can give valuable clues as to the manner and cause of the accident. In diving accidents, identifying the cause of the accident may contribute to improving diving safety by providing additional information to existing information in terms of diving safety. The depth at which the body was found may not be the depth at which the death event occurred, which developed by drowning in majority of the accidents. In 85-90% of drowning cases some fluid aspiration to the lungs is mentioned, but there is no aspiration in 10-15% of cases, the amount of fluid aspirated to the respiratory tract and lungs is known to differ from one person to another (7, 8, 9). Diatoms may be one of the most important diagnostic tools used in the cases of drowning (10, 11). Planktonic organisms detected in internal organs can provide valuable clues as to whether the cause of death of bodies found in water was drowning, the site and depth of drowning, and are used by experts to elucidate death and its mechanism (12, 13, 14, 15, 16).

In this study, the presence of planktonic organisms in the lungs of rats drowning at surface and at a depth of 30 meters was investigated and its value in determining the depth of drowning was discussed.

2. Materials and Methods

The preliminary study was conducted using samples taken from Kaşıkçı Island Region, and the main study was conducted with samples taken from the Tuzla Coast ,in the Marmara Sea. Two liters of seawater from the surface and 30 meters depth were taken for preliminary study and 60 liters of seawater were taken from the surface and 30 meters depth for the study using Nansen bottle (Figure 1). Lugol solution was added to the water samples to increase the visibility of chloroplasts (17, 18).



Figure 1. Sampling station in the Marmara Sea

16 Wistar Albino rats were divided into two groups as Group-1 and Group-2 and each group included eight rats. The rats in Group-1 and Group-2 were suffocated in 38x25x15 cm size plastic cages by being submerged in a plastic container with the size of 60x40x24 cm. containing seawater sample taken from the surface at 1ATA pressure (to simulate the conditions of liquid aspiration occurring at 1 ATA environmental pressure at surface) and seawater sample taken from the depth of 30 meters at 4ATA pressure (to simulate the conditions of liquid aspiration at 4 ATA environmental pressure at the depth of 30 meters) respectively. The pressurized environment was provided in the double lock multiplace chamber at the Department of Undersea and Hyperbaric Medicine, Istanbul Faculty of Medicine, Istanbul University.

An autopsy was performed on the rats about two hours after the drowning. The abdomen of the rats was fully opened, with the incision opening just below the xiphoid in the supine position, widening to both sides. The skin incision is extended upwards, with the skin grazed on both sides in the thoracic and cervical areas. The diaphragm was cut from the rib compound so that both lung bases were protected and the thorax cavity was reached from the bottom. The sternal lid was then removed, including the manubrium sternum, by cutting through both parasternal lines with scissors. The amount of fluid in both chest cavities was evaluated as macroscopically and the trachea separated from the esophagus by blunt dissection was connected at 2 levels over 0.5 cm of bifurcation and cut between the connecting points and separated from the larynx. Blunt dissection was continued and the lungs were enclosed in a clean petri dish along with the trachea. After macroscopic examination, each lung tissue was separated into small particles (approximately 0,5x0,5 mm) with the help of a dermatome knife and put into bottles containing 2 cc 10% formaldehyde solution with a volume of 10 ml.

The lung tissue, which was cut into pieces and preserved in 10% formaldehyde, was examined in the plankton research laboratory of the Department of Hydrobiology, Department of Biology, Faculty of Science, Istanbul University. The seawater samples to be examined was subjected to a process of precipitation for a week, then the excess water at surface was removed by siphoning. 4% formaldehyde was added to the part at the bottom that was thought to be rich by planktonic organisms. The identification of the species was made under a phase-contrast inverted microscope.

Ethical Declaration

This article was composed by reorganization of the specialty thesis entitled as "Farklı Derinliklerde Boğulan Sıçanların Akciğerlerde Tespit Edilen Planktonik Organizmalar Yardımıyla Boğulma Derinliği Tespit edilebilir mi?"

3. Results

Rats in both groups drowned about 3 minutes after immersion. During the autopsy performed two hours after the drowning, macroscopic examinations showed that the lungs of rats drowned at 4 ATA pressure had a greater volume and there were hemorrhaging foci on the surface of the lung. In rats drowned at 1 ATA pressure had smaller lungs in volume compared to the other group and there were petechial hemorrhages present on the lung surface (Figure 2).



Figure 2. The lungs of rats drowned at 1 ATA (A) and 4 ATA (B) pressures.

22 phytoplankton (Figure 3) and 5 zoo planktonic taxon were identified in the seawater sampled from the surface, while only 7 phytoplankton taxa were found in the seawater sample taken from 30 meters depth. Three of the diatoms detected were found in both seawater samples (Table 1). As a result of the examination of the fragmented lungs stored in formaldehyde solution, no plankton species were found.

Table 1. Planktonic organisms detected in the seawater samples taken in the preliminary and main study.

Taxa	Surface	30 m depth
PYHTOPLANKTON		
Dinophyceae		
Alexandrium minutum Halim, 1960	*	
Dinophysis caudata Saville-Kent, 1881	*	
Kofoidinium velleloides Pavillard,1929	*	
<i>Noctiluca scintillans</i> (Macartney) Kofoid and Swezy,1921	*	
<i>Phalacroma rotundatum</i> (Claparéde and Lachmann) Kofoid & Michener, 1911 and Michener	*	
Prorocentrum micans Ehrenberg, 1834	*	

Protoperidinium divergens (Ehrenberg)	*	
Balech, 1974		
Tripos fusus (Ehrenberg) F.Gómez, 2013	*	
Bacillariophyceae		
<i>Cerataulina pelagica</i> (Cleve) Hendey, 1937	*	
Chaetoceros sp.	*	*
Coscinodiscus radiatus Ehrenberg, 1840	*	
Coscinodiscus sp.	*	
Cylindrotheca closterium (Ehrenberg) Reimann & J.C.Lewin, 1964	*	
<i>Dactylioselen fragilissimus</i> (Bergon) Hasle, 1996	*	
<i>Ditylum brightwellii</i> (T.West) Grunow, 1885	*	
<i>Guinardia flaccida</i> (Castracane) H.Peragallo, 1892	*	
<i>Hemialus hauckii</i> Grunow ex Van Heurck, 1882		*
Leptocylindrus danicus Cleve, 1889	*	
Proboscia alata (Brightwell) Sundström, 1986	*	*
<i>Pseudo-nitzschia pungens</i> (Grunow ex Cleve) G.R. Hasle, 1993	*	
Pseudosolenia calcar-avis (Schultze) B.G. Sundström, 1986 (=Rhizosolenia calcar-avis Schultze)		*
Rhizosolenia hebetata Bailey, 1856		*
Rhizosolenia setigera Brightwell, 1858	*	*
<i>Skeletonema costatum</i> (Greville) Cleve, 1873		*
Thallassiosira sp.	*	
Euglenophyceae		
<i>Eutreptiella</i> sp.	*	
ZOOPLANKTON		
Ciliophora		
Ciliate	*	
Amphorellopsis tetragona (Jörgensen)	*	
Kofoid and Campbell, 1929		
Cladocera		
Penilia avirostris Dana, 1949	*	
Apendicularia		
<i>Oikopleura</i> sp.	*	
Copepoda		
Copepoda nauplii	*	
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Figure 3. Some phytoplankton species found in seawater samples (scale=20 µm)

4. Discussion

Crime scene investigation, witness statements, macroscopic findings detected in autopsy, biological evidence and detailed toxicological analysis are utilized in forensic investigations and planktonic organisms contribute significantly to the clarification of the cause and mechanism of death in bodies recovered from water (13, 19).

Diatom detection has been used for over 50 years in the diagnosis of drowning (20). In a study conducted in Canada, 771 cases of drowning were retrospectively examined. 738 of these cases were drowned in fresh water and 205 of these cases had diatoms detected in the bone marrow and there was a correlation between the diatom intensity in the drowning environment and the results of the diatom test that increased the reliability of the diatom test (21). In another study, 81 out of 133 cases drowning were examined by sampling from the water where drowning occurred, and diatoms were found in 70 samples. However, of the 70 patients who drowned in the water sample with diatoms, it was not possible to detect diatoms in the blood and tissues of 51 cases, while it was possible in 19 cases. (22). When investigating a drowning incident, the greater the concentration of plankton in the event zone, the more likely the diatom test will be positive.

In a study of our country, a diatom examination carried out during an autopsy on one of the three diving accidents that resulted in death brought to the mortuary department of Istanbul Forensic Medicine Institute, more than 20 diatoms in the lungs, 1-2 diatoms in the liver, 1-2 diatoms in the brain and 3-4 diatoms in the bone marrow have been detected. It was concluded that diver died as a result of liquid aspiration due to the characteristic similarity of diatoms in lung tissue preparations and in other tissue preparations (23).

Within a 5-year period covering 2003-2007 in the mortuary department of Forensic Medicine, diatom analysis yielded negative results in 300 of the 433 drowning cases that were autopsied in Istanbul and the negative rate was found to be around 70% in total. This rate was found to be higher in sea water cases (72.5%) than in freshwater cases (58.1%) (24).

Water samples should be taken immediately after the accident while the planktonic organisms are investigated to give an idea of the depth at which the drowning occurred. If water samples are taken at different times, or if the body is displaced for any reason after drowning, it should be kept in mind that the planktonic organisms that will be found in the lungs may not be similar to the planktonic organisms in the water sample.

In this study, no samples of plankton were found in the lungs of rats that were drowned in water samples taken from different depths. This can be attributed to the low density of plankton. In addition, the duration of immersion can also be considered as an important factor. In this experimental study, drowning occurred within about 3 minutes and the duration of immersion of rats is limited. In many cases of drowning, a long time passes until the body is found. In addition, when compared to the small lung capacity of rats, it is obvious that both the amount of water aspirated and the planktonic organism would be much more likely to enter the lungs in a person with a very large lung capacity.

It is possible to determine whether planktonic organisms that migrate to different depths at different times of the day can be used in determining the depth at which the drowning occurs, by finding out whether different species live at different depths. For this purpose, it is important to have a database of planktonic organisms in samples taken from different depths at different times in the regions to be examined.

Although the diatom test may be controversial, the most important issue is whether diatoms reach the organs in bodies that remain in water postmortem or not. In a study conducted in Eskişehir, one of the 10 rats who died out of water and were kept in water postmortem for 24 hours, 7 diatoms in the lung of 1 rat, 1 diatom in the lung of 1 rat, 1 diatom in bone marrow of 1 rat were detected, while no diatom was detected in the remaining 7 rats. In the lungs of 4 of the 10 rats that were kept in water postmortem for 72 hours, 1, 7, 9 and 10 diatoms were

detected, while there is no diatoms were detected in any organ of the remaining 6 rats. However, diatoms were detected in the lungs and other tissues of all 10 rats that were drowned, and the number of diatoms were higher in the lungs of these rats than in other groups. As a result of this study, it was stated that the diatoms detected in the organs of drowning cases were numerically greater than the diatoms detected in bodies that remained in water postmortem, and diatoms could be found in more organs (25).

Diatoms are photosynthetic algae that are of great importance in biological, ecological and economic terms and can live in any kind of aquatic environment. The most typical feature of the diatom cell is that it has a box-like exoskeleton called frustule=theca, made of more or less durable, hydrated silicate. The fragile yet durable structure of the silica skeleton allows it to be fossilized, and because of this silica wall, it has been known for many years that they make remains in sea sediments (26, 27). Although dinoflagellates are dominant in terms of species diversity in the seas, diatoms are more preferred in forensic studies than other groups in cadavers due to their durable structure due to their silica walls.

All these evaluations mentioned above require a multidisciplinary approach involving various experts. Such a team should include experts with technical knowledge who can evaluate the body and the outbuildings of the body in detail, police divers who will conduct criminal investigation at the crime scene, forensic experts, pathologists, toxicologists and hydrobiologists who will provide some laboratory supports to examine the body.

5. Conclusion

Although detection is not possible in every case, diatoms in organs is a valuable finding for the diagnosis of cause of deaths in drowning. Knowing the depth at which the drowning occurred will contribute significantly to the determination of the manner in which the diving accident occurred. Comparing the diatoms found in water samples taken from certain depths at the time of the accident with the diatoms found in the lungs of the casualty may give an idea of the depth of drowning. A database to be created on the daily and seasonal distribution of the depth of diatoms in the seas and inland waters may help determining the depth of drowning if there are diatoms in the organs.

Limitations of the Study:

The study should be tested using different extraction methods for the detection of planktonic organisms in aspiration fluid and lung tissue and planktonic organisms content dense waters. Detection of planktonic organisms in water samples will increase the reliability of the experiment by repeating it before the experiment

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